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## ENTOMON

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## Molecular variation in the natural populations of pink bollworm, *Pectinophora gossypiella* (Saunders)

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**ABSTRACT:** *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) populations, collected from eight different cotton fields in districts of southern Tamil Nadu, India were studied to determine their genetic variation using the technique of RAPD-PCR. Out of the twenty primers tested, primer Kit A09 and A20 yielded clear, consistent and discrete scorable banding patterns. The UPGMA based dendrogram of the two primers grouped the populations of *P. gossypiella* in two clusters. The similarity coefficients ranged from 0.03 to 0.86, indicating that no two populations were 100% similar. The similarity matrix of all the primers indicated that most of the populations exhibited coefficient <50%. This suggested that the populations of *P. gossypiella* exhibit a great degree of intra-species variation. © 2007 Association for Advancement of Entomology

**KEYWORDS:** *Pectinophora gossypiella*, genetic diversity, RAPD-PCR

### INTRODUCTION

Molecular characterization of insects is frequently used as a tool to identify the intra-species variations of insects. Insects such as *Listronotus banariensis* (Williams *et al.*, 1994), *Ceratitis capitata* (Haymer, 1994), *Choristoneura* (Deverno *et al.*, 1998), *Helicoverpa armigera* (Zhou *et al.*, 2000), *Bemisia tabaci* (Moya *et al.*, 2001), *Cochliomyia* sp. (Skoda *et al.*, 2002), *Cylas formicarius* (Kawamura *et al.*, 2002) have been subjected to such studies and the presence of wide variation within species has been reported. Such studies were also carried out in India by many workers. Amudha *et al.* (2000) reported that there are RAPD markers linked to *Nilaparvata lugens*. Karthikeyan *et al.* (2005) have attempted to clarify PCR-based DNA patterns in *Leucinodes orbonalis*. The pink bollworm, *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) a serious pest of cotton all over India and subjected to intensive

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exposure to insecticides is likely to show significant DNA polymorphism. This aspect has not been studied so far and hence the work reported in the paper.

DNA markers are effective tools in making inferences about movement between insect populations, since they present selectively unbiased characters (Black *et al.*, 2001). Randomly amplified polymorphic DNA (RAPD) is a polymerase chain reaction (PCR) technique that allows detection of many polymorphisms within the genomic DNA in a short time (Welsh and McClelland, 1990; Williams *et al.*, 1994). The RAPD technique has been widely used to elucidate the geographical origin and gene flow among insect populations (Ayres *et al.*, 2003). The properly performed RAPD analysis is a useful and reliable tool for studying the ecology and genetic structuring of populations (Armstrong and Wratten, 1996; Brown *et al.*, 1997; Pearson *et al.*, 2002). In the present investigation, the field populations of *P. gossypiella* collected from different cotton fields of Southern Tamil Nadu was analyzed for their genetic variation based on their RAPD banding pattern.

#### MATERIALS AND METHODS

The fifth instar larvae of *Pectinophora gossypiella* from infested cotton bolls were collected from cotton fields in eight different districts of Southern Tamil Nadu, India. Genomic DNA was isolated using phenol-chloroform procedure (Ballinger-Crabtree *et al.*, 1992). DNA (20 ng) was dissolved in 20  $\mu$ l of PCR reaction buffer containing 10 mM Tris-HCl (pH 9.0), 1.5 mM MgCl<sub>2</sub>, 50 mM KCl, 0.01% gelatin, 0.2 mM dNTPs, 20 pM of primer and 0.5 U of DNA polymerase. Twenty primers (RAPD Kit A01 to A20) obtained from Integrated DNA Technologies (IDT), USA were used for RAPD-PCR studies. PCR was done according to the methods of Williams *et al.* (1990) with initial heat step (94 °C for 5 min), 40 cycles of denaturation (94 °C for 1 min.), annealing (36 °C for 1 min), extension (72 °C for 2 min) and final extension step (72 °C for 7 min). Amplification was performed using a programmable thermal cycler PTC-150 (MJ Research, USA). The products of PCR and DNA size markers [ $\lambda$  DNA digested with *Eco*RI and *Hind* III (Bangalore Genei)] were loaded onto a 1.6% tris-borate-EDTA (Sambrook *et al.*, 1989) agarose gel and run for 4 h at 50 V. The gels were stained with ethidium bromide and photographed. RAPD profiles were subjected to gel documentation system (Vilber-Lourmat, France). Dendrogram and similarity index (Unweighted Pair Group Method with Arithmetic Mean - UPGMA) were constructed using Bioprofile 1D software).

#### RESULTS AND DISCUSSION

Of the primer set tested, primer KitA09 and A20 yielded clear, consistent and discrete scorable banding patterns for the populations of *P. gossypiella* (Figs. 1 and 2). The UPGMA based dendrogram of both the primers grouped the populations of *P. gossypiella* in two clusters. The similarity coefficient values ranged from 0.03 to 0.86, indicating that most of the populations exhibited coefficient <50%. This

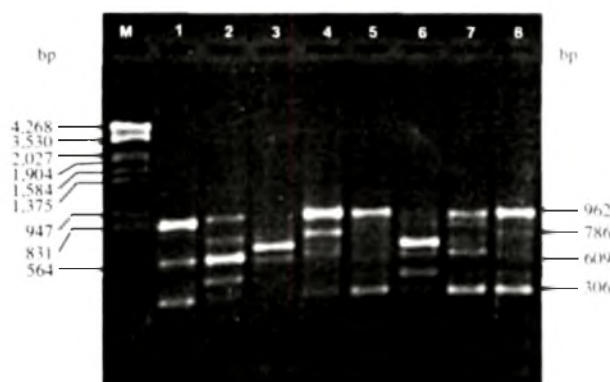


Fig. 1a

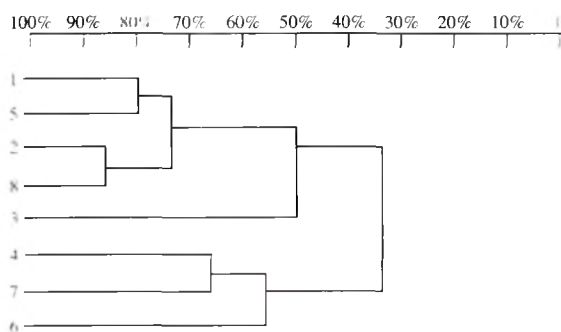


Fig. 1b

FIGURE 1. (a) Random amplified polymorphic DNAs of different populations of *Pectinophora gossypiella* generated by the Primer RAPD Kit A9. (b) Dendrogram showing diversity of the populations of *P. gossypiella* as revealed by the Primer RAPAD Kit A9. M, Molecular marker; 1, Madurai population; 2, Sivaganga population; 3, Virudhunagar population; 4, Theni population; 5, Dindigul population; 6, Ramanathapuram population; 7, Tirunelveli population; 8, Tuticorin population

suggested that all the populations of *P. gossypiella* exhibit a great degree of intra-species variation.

Figure 1a shows the RAPD profile of *P. gossypiella* generated by the primer KitA09. The scorable fragments were produced by KitA09 primer with the molecular weight range of 306bp to 962bp. The RAPD profile showed a maximum of five fragments in Sivaganga population and a minimum of two fragments in Virudhunagar population. The UPGMA dendrogram showed two major clusters, one comprised populations of Madurai, Dindigul, Sivaganga, Tuticorin and Virudhunagar and the other included Theni, Tirunelveli and Ramanathapuram populations (Fig. 1b).

The RAPD profile of *P. gossypiella* generated by the primer KitA20 produced amplified fragments of DNA in the molecular weight ranging 254bp to 1353bp

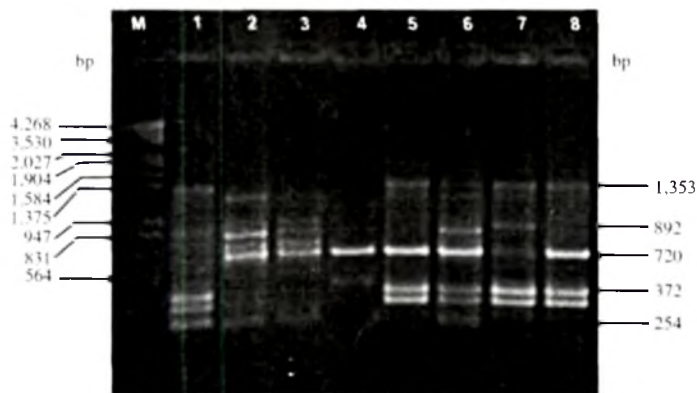


Fig. 2a

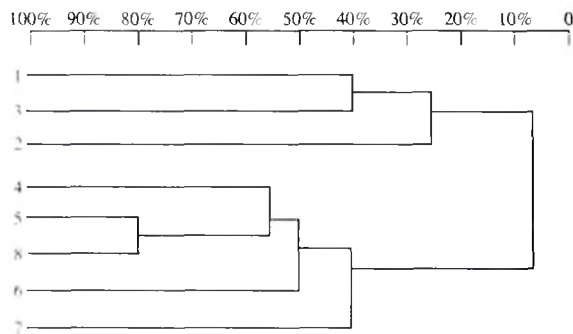


Fig. 2b

FIGURE 2. (a) Random amplified polymorphic DNAs of different populations of *Pectinophora gossypiella* generated by the Primer RAPD Kit A20. (b) Dendrogram showing diversity of the populations of *P. gossypiella* as revealed by the Primer RAPAD Kit A20. M, Molecular marker; 1, Madurai population; 2, Sivaganga population; 3, Virudhunagar population; 4, Theni population; 5, Dindigul population; 6, Ramanathapuram population; 7, Tirunelveli population; 8, Tuticorin population

(Fig. 2a). A maximum of eight numbers of amplified fragments was recorded in the Sivaganga population and only two fragments were recorded in Theni population. The UPGMA based dendrogram (Fig. 2b) generated from the profile clearly indicated two major clusters that comprised populations of Madurai, Sivaganga and Virudhunagar in one group and Theni, Tuticorin, Ramanathapuram and Tirunelveli in another group.

Diverse kinds of molecular markers are capable of revealing different levels of genetic variation, making population genetics studies possible on a wide range of geographical scales (Haymer, 1994). RAPD markers are generated by the amplification of random DNA segments with single primers of arbitrary nucleotide sequence. Most polymorphisms are inherited as dominant traits, and are detected as the presence or

absence of amplification products from a single locus (Williams *et al.*, 1994). Usually for all molecular genetic diversity studies random or arbitrary primers are preferred (Black *et al.*, 2001). As these are non-specific, many primers are used for a single reaction or sample. Out of many different primers used a primer or few primers generating clear, consistent, discrete scorable fragments are chosen as DNA markers for a particular species. There is also no standard procedure available in using specific primers for RAPD analysis. In the current study twenty different primers were selected to produce markers for the populations of *P. gossypiella* and only two primers gave most consistent and scorable products.

In the present investigation, the field populations of *P. gossypiella* collected from different cotton fields of Southern Tamil Nadu was analyzed for their genetic variation based on their RAPD banding pattern. The important observation made out of the study is that all the populations in the sub-clusters showed a similarity of 50–86% for the primer kit A09 and more than 25% for the primer kit A20 indicating the primer kit A20 revealed much intra-species variation. The RAPD analysis of eight populations revealed that no two populations are 100% similar suggesting high genetic variability among populations. On the basis of this study it is concluded that the South Tamil Nadu populations of *P. gossypiella* are genetically heterogeneous.

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## Effect of temperature on development of *Lipaphis erysimi* (Kaltenbach) (Homoptera: Aphididae)

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**ABSTRACT:** Laboratory studies were carried out on the development of *Lipaphis erysimi* at 15°, 20°, 22.5°, 27°, 30° and 32 °C on detached shoot of mustard plant. The development period from first instar nymph to adult formation was the longest (16.33 d) at 20 °C and shortest (1.66 d) at 32 °C. Nymph mortality was 66.66 percent at 32 °C and 4.83 percent at 20 °C. The highest offspring (39.85 nymphs/female) was produced at 20 °C. The maximum intrinsic rate of increase ( $r_m$ ) (0.341 nymphs/female/day) was obtained at 30 °C whereas  $r_m$  was negative (−0.287) at 32 °C. Reproduction rate ( $R_o$ ) was highest at 20 °C (9.195), and lowest at 32 °C (0.365). The shortest generation time ( $\tau$ ) was found at 32 °C.

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**KEYWORDS:** *Lipaphis erysimi*, mustard, fecundity, intrinsic rate of increase, reproduction rate, generation time

### INTRODUCTION

*Lipaphis erysimi* (Homoptera: Aphididae) is a key pest of rapeseed-mustard and yield loss is considerably high on *Brassica* crops (Bakhetia, 1984). Weather conditions play an important role in the appearance and intensity of *L. erysimi* (Sinha *et al.*, 1990). Temperature affects the rate of development, reproduction and survival of aphids (Asante *et al.*, 1991). Construction of life table is an important component in the understanding of population dynamics of a species (Carey, 1993). Although insects are not always subjected to constant temperatures in nature; a controlled study can provide valuable insight into population dynamics of a particular species (Summers *et al.*, 1984).

Essential information on the effect of temperature on development and fecundity of *L. erysimi* is lacking. Hence studies on the development and reproduction of the insect in the laboratory under controlled conditions were carried out.

\*Corresponding author

TABLE 1. Effect of temperature on the development of nymphs of *L. erysimi*

Temperature °C	Nymphs <i>n</i>	Nymph mortality (%) <i>d<sub>x</sub></i>	Development time (d) <i>x</i>
15.0	46	15.71	06.00 ± 0.58
20.0	58	04.83	16.33 ± 0.88
22.5	50	09.68	09.00 ± 1.00
27.0	42	21.77	04.00 ± 0.58
30.0	75	30.55	03.33 ± 0.33
32.0	52	66.66	01.66 ± 0.33
LSD ( <i>P</i> = 0.05)		0.189*	2.167*
Correlation		0.829*	-0.829*

## MATERIALS AND METHODS

*L. erysimi* was reared on detached shoots of mustard plant (*Brassica juncea* (L.) Czern and Coss), Alankar variety at  $25 \pm 1^\circ \text{C}$ ,  $75 \pm 5$  percent relative humidity and photoperiod of 8: 16 (L: D) h with artificial light using 16 W bulb in a B.O.D. chamber for 5 generations before the experiments began. Apterous adult aphids collected from the culture within 24 h after emergence were used for the experiment. Single aphid was placed on one mustard shoot which was then placed in a plastic cage (15 cm) and then kept in B.O.D. incubator maintained at  $15^\circ$ ,  $20^\circ$ ,  $22.5^\circ$ ,  $27^\circ$ ,  $30^\circ$  and  $32^\circ \text{C}$  at humidity and photoperiod levels mentioned earlier. The aphids were checked daily for exuviae and survivorship at all temperature regimes. Aphids were transferred to new shoot at every third and fourth day till the death of the test aphids. The number of offspring/female and mortality were determined daily. Nymphs were removed from the test chamber after counting and these observations continued until the mature aphid died. Life table was constructed by the method of Southwood (1978), data were analyzed by analysis of variance (ANOVA) and difference was determined by the least significant difference (LSD) test and correlation was calculated by Pearson method.

## RESULTS AND DISCUSSION

### Development of nymph

Temperature has significantly ( $P < 0.05$ ) influenced mortality and development of nymph of *L. erysimi* (Table 1). Mortality is significantly correlated ( $r = 0.829$ ,  $P < 0.05$ ) with temperature. Nymph mortality was highest (66.66%) at  $32^\circ \text{C}$  and lowest (4.83%) at  $20^\circ \text{C}$ . The development time from first instar to formation of adult is considerably decreased with increase of temperature from  $20^\circ$  to  $32^\circ \text{C}$  ( $P < 0.05$ ) as well as below  $20^\circ \text{C}$  showing a negatively significant correlation ( $r = -0.829$ ,  $P < 0.05$ ).

TABLE 2. Effect of temperature on the life table indices of *L. erysimi*

Temperature °C	Total aphids <i>n</i>	No. of offspring/♀	Intrinsic rate (♀♀/♀/day) <i>r<sub>m</sub></i>	Reproduction rate (♀♀/♀) <i>R<sub>o</sub></i>	Generation time <i>τ</i>
15.0	43.66	31.465 ± 1.212	0.252 ± 0.002	8.553 ± 0.055	8.51 ± 0.021
20.0	55.00	39.850 ± 1.026	0.261 ± 0.002	9.195 ± 0.001	8.50 ± 0.021
22.5	48.66	29.940 ± 1.167	0.229 ± 0.001	7.027 ± 0.004	8.50 ± 0.006
27.0	50.00	16.870 ± 0.624	0.128 ± 0.002	2.613 ± 0.005	7.50 ± 0.012
30.0	65.33	34.520 ± 0.563	0.341 ± 0.001	6.546 ± 0.001	5.50 ± 0.020
32.0	55.00	02.250 ± 0.110	-0.287 ± 0.002	0.365 ± 0.003	3.51 ± 0.017
LSD ( <i>P</i> = 0.05)		2.92*	0.018*	0.068*	0.0535*
Correlation		-0.543 <sup>ns</sup>	-0.371 <sup>ns</sup>	-0.886*	0.986**

Significant at \*\*0.01 and \*0.05

<sup>ns</sup> Non-significant

### Development and reproduction

Figure 1 shows that female *L. erysimi* starts producing nymphs within 24 h of its emergence and as long as adult survives ( $P < 0.05$ ). Temperature caused a pronounced effect on the birth of young ones ( $P < 0.05$ ) while erratic non-significant correlation was obtained with age but negatively significant correlation was calculated at 27° C ( $r = -0.854$ ,  $P < 0.05$ ). Number of offspring produced was less in the beginning of age and then increased with age and decreased to zero on the last day of life at 10° and 22.5 °C but 11.1 nymphs were born at the age of day fifth at 30 °C. The highest birth occurred on 5-day at 15 °C. However, total number of offspring/female is negatively correlated (non-significant,  $r = -0.543$ ,  $P > 0.05$ ) with temperature (Table 2); 39.85 nymphs/female were born at 20 °C and 2.25 at 32 °C. Reproduction rate ( $R_o$ ) significantly differed with temperature ( $P < 0.05$ ) that caused an inhibitory effect on the production of young ones ( $r = -0.886$ ,  $P < 0.05$ );  $R_o$  was highest at 20 °C (9.195) and lowest (0.365) at 32 °C.

Intrinsic rate of increase ( $r_m$ ) was also significantly affected by temperature ( $P < 0.05$ ) but negatively correlated (non-significant) ( $r = -0.371$ ,  $P > 0.05$ ).  $r_m$  was negative (-0.287) at 32 °C and highest at 30 °C (0.341) (Table 2). At 20 °C,  $r_m$  was greater than at 15° and 22.5 °C. The generation time ( $\tau$ ) also differed significantly ( $P < 0.05$ ) as well as correlated significantly ( $r = 0.986$ ,  $P < 0.01$ ) and was the shortest at 32 °C.

The present study shows that temperature has significant influence on the mortality and development of nymphs of *L. erysimi*. At 32 °C nymph mortality was 66.66 percent and the development period was also shorter (1.66 day) which considerably inhibited the population growth of *L. erysimi* ( $r_m = -0.287$ ). The same result is reported by Wang and Tsai (2000) in *Aphis spiraecola*, that high temperature (32 °C) caused a decrease in development rate, caused high mortality (71%) of immature stages, and lower progeny production, resulting in a much lower  $r_m$  (0.010). They

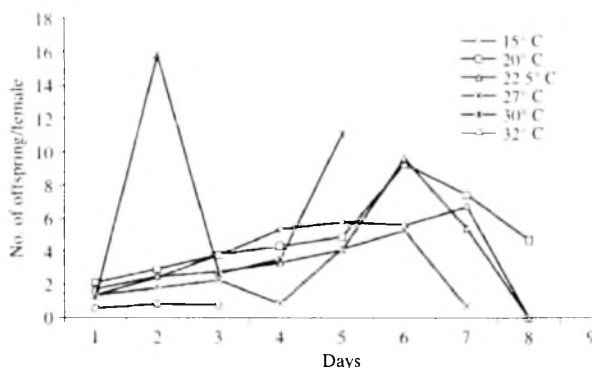


FIGURE 1. Effect of temperature on offspring/female of *L. erysimi*

also found that *A. spiraecola* failed to survive at 35 °C. Results of Satar and Yokomi (2002) on *Brachycaudus schwartzi* showed that nymph mortality was lowest at 20° (1.8%) and increased to 28 percent at 30 °C, and 100 percent at 35 °C which was considered as the upper threshold. The development time of nymph decreased from 19.9 d at 15 °C to 8.9 d at 25 °C and then began to increase from 27.5 °C.

Intrinsic rate of increase ( $r_m$ ) of different aphid species varied significantly with temperature and is in the range of 0.3–0.4 (Briese, 1988). We found that the highest  $r_m$  (0.341) occurred at 30 °C with a higher reproduction rate ( $R_o = 6.546$ ) as well as higher total progeny production (34.52) with short generation time. Wang and Tsai (2000) obtained the highest  $r_m$  (0.308) at 25 °C in *A. spiraecola* and explained that because of its faster development as well as considerably higher rate of daily progeny production and greater total progeny production, significantly faster development of immature stages and a shorter reproductive period, the mean generation time was considerably shorter. Wang *et al.* (1997) reported negative  $r_m$  values at extreme temperatures of 10° and 35 °C, which caused a prolonged development and reduced survivorship of immature stages as well as reduced fecundity in *A. nasturtii*. Similar adverse effect was also found by us at 32 °C where  $r_m$  was negative.

In the present study, the reproductive rate ( $R_o$ ) is highest at 20 °C (9.195) and lowest (0.365) at 32 °C. Similar results were also reported by Satar and Yokomi (2002) that  $R_o$  was highest at 20 °C (43.5 nymphs/female) in *B. schwartzi*. Wang and Tsai (2000) found highest  $R_o$  (41.0) at 20 °C which declined considerably at 25 °C in *A. spiraecola*.

In the present study, the generation time ( $\tau$ ) was shortest at 32 °C. However, Satar and Yokomi (2002) found shortest generation time at the warmest temperatures of 27.5° and 30 °C but temperatures between 22.5° and 25 °C are favorable for *B. schwartzi*. A prolonged generation time of 40.3 d and shortest of 8.3 d was found at 10° and 35 °C, respectively, in *A. nasturtii* (Wang *et al.*, 1997).

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## Consumption and utilization of different genotypes of rapeseed-mustard by larvae of *Pieris brassicae* (Linnaeus)

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**ABSTRACT:** Twelve genotypes of rapeseed-mustard belonging to *Brassica napus* (GSL 1, GSL 2, PGSH 51, PGSH 124), *B. juncea* (Purple mutant, NDRC 190, YSRLC 9-26, RL 1359, PBCM 1159), *B. campestris* (TL15, BSH 1) and *B. carinata* (PC 5) with diverse characters were selected and consumption and utilization indices of *Pieris brassicae* (Linnaeus) were assessed. The consumption index (CI), growth rate (GR), efficiency of conversion of ingested food (ECI), approximate digestibility (AD) and efficiency of conversion of digested food (ECD) varied from 2.89 to 1.52, 0.65 to 0.39, 67.6 to 50.2, 22.7 to 16.6 and 46.5 to 35.1%, respectively. The CI, GR, AD and ECI decreased gradually with increase of age of larvae and the ECD did not vary significantly. All the indices varied significantly on different genotypes. Lower CI, higher GR, AD and ECD of larvae fed on PC 5, GSL 1 and PGSH 51 indicated these as most susceptible whereas PBCM 1159 and BSH 1 with higher CI and lower GR, AD and ECD were rated as tolerant genotypes for development of *P. brassicae*.

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**KEYWORDS:** *Pieris brassicae*, cabbage butterfly, consumption and utilization indices, rapeseed-mustard

### INTRODUCTION

Host plant resistance is one of the major components of integrated pest management (IPM). Continuous efforts are being made to select genotypes of food plants resistant to insect pests. The resistant genotypes are screened based on ovipositional preference, feeding preference, tolerance of plants and growth and development of pest on particular genotype. The quantitative aspects of insect nutrition like, digestibility and efficiency of conversion of food are reported to be affected by the quality of food. It is also known that consumption and utilization indices of food by insect pests differ

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significantly on resistant and susceptible genotypes (Kaushal and Vats, 1983; Chibber *et al.*, 1985).

Rapeseed and mustard are the important oilseed crops of India. These crops are attacked by more than 40 species of insect pests and among them the cabbage butterfly, *Pieris brassicae* (Linnaeus) has emerged as a serious pest which inflicts up to 92.2 per cent loss in seed yield (Anonymous, 1996). Keeping these points in view, the present studies were undertaken with the aim of identifying tolerant and susceptible genotypes of rapeseed-mustard, based on the consumption and utilization indices of food by *P. brassicae*.

#### MATERIALS AND METHODS

Twelve genotypes of rapeseed-mustard belonging to *Brassica napus* (GSL 1, GSL 2, PGSH 51, PGSH 124), *B. juncea* (Purple mutant, NDRC 190, YSRLC 9-26, RL 1359, PBCM 1159), *B. campestris* (TL15, BSH 1) and *B. carinata* (PC 5) were sown in earthen pots and maintained in Entomology Farm, Punjab Agricultural University, Ludhiana. The genotypes were sown periodically in pots to ensure regular supply of food during the conduct of experiments. The newly hatched larvae of uniform age, collected from field and reared in laboratory, were transferred on fresh leaves of the test genotypes in different glass jars. As soon as these larvae entered second instar, the larval weight was taken and transferred to plastic vials measuring 31 mm in diameter and 100 mm in height. Ten larvae represented one replication and there were four replications for each treatment and the experiment was repeated twice. The weighed quantity of food was given in plastic tubes to the larva and after 24 h the larval weight, weight of unconsumed food and weight of faecal matter were recorded. The tubes were cleaned and weighed and fresh food was again provided. The process was continued till the larva entered the prepupal stage and stopped feeding. Separate set of tubes containing weighed leaves was placed in the same environment, which served as control to record the moisture loss. Consumption index (CI), growth rate (GR), efficiency of conversion of ingested food (ECI) approximate digestibility (AD) and efficiency of conversion of digested food (ECD) were calculated for second to fifth instar for all the test genotypes by method suggested by Waldbauer (1968). The data were analyzed using completely randomized block design.

#### RESULTS AND DISCUSSION

##### **Consumption and utilization of food by different instars of *P. brassicae***

Data are presented in Table 1. Consumption Index (CI), was highest in second instar larvae and it decreased with increase in age of larvae. The decreasing trend in growth rate (GR) from second to fifth instar larvae showed that growth of larvae was fast during early instars and the growth rate slowed down in later instars. Similarly the highest AD was recorded in second instar and lowest in fifth instar. Like CI and GR it also decreased with increase in age of larvae. The efficiency of conversion of ingested food (ECI), which measures the insect's ability to utilize the ingested food for its

TABLE 1. Consumption and utilization indices of different larval instars of *Pieris brassicae* on rapeseed-mustard

Larval instar	Indices				
	Consumption index (CI)	Growth rate (GR)	Approximate digestibility (AD)	Efficiency of conversion of ingested food (ECI)	Efficiency of conversion of digested food (ECD)
2nd	2.89	0.65	67.6 (55.29)	22.7 (28.45)	37.4 (37.69)
3rd	2.58	0.57	62.9 (52.47)	21.4 (27.51)	39.8 (39.07)
4th	2.07	0.48	55.3 (48.06)	19.5 (26.18)	41.5 (40.10)
5th	1.52	0.39	50.2 (45.11)	16.6 (24.09)	40.4 (39.46)
CD ( $p = 0.5$ )	0.26	0.03	(1.12)	(0.30)	(NS)

Figures in parentheses are *arc sine* transformed values.

growth, also follows the same trend as CI, GR and AD. Waldbauer (1968) reported that ECI and AD showed declining trend in five instars of *Bombyx* spp. Gupta and Maleyvar (1981) and Sharma *et al.* (1999) reported gradual decline of ECI in *P. brassicae* with age advance. On the other hand, the efficiency of conversion of digested food (ECD), varying from 37.4 to 41.5%, did not show statistical significance. Kaushal and Vats (1983) and Sharma *et al.* (1999) also reported that ECD of *P. brassicae* grown on cauliflower did not vary significantly with reference to the age of larva.

#### Comparative tolerance/resistance of different genotypes of *Brassica* spp. to the larvae of *P. brassicae*

The data are shown in Table 2. The consumption indices of GSL1 (1.65) was the least and it came on par with PC5 (1.78) and they were followed by PGSH51 (1.87) and GSL2 (1.90), which were also on par. The remaining genotypes in which CI ranged from 2.24 to 2.34 came on par and the highest indices observed in the genotypes PBCM1159 and BSH1 with CI of 2.48 and 2.34, respectively, were on par. Genotypes with higher consumption indices may show more leaf damage in comparison with the remaining genotypes, but higher intake of food indicates these plants are nutritionally inadequate due to which more intake of food became essential to fulfill the nutritional requirements of the insect (Waldbauer, 1968). Based on this criterion, PBCM1159 and BSH1 with higher CI, were categorized as tolerant and GSL1, PC5, PGSH51 and GSL2 with lower CI, as most susceptible and remaining ones as of intermediate tolerance to *P. brassicae*. With reference to the indices of growth rate (GR), PC5 (0.57), GSL1 (0.56) and PGSH51 (0.55) came on par and highest in rank, which indicated these as most susceptible genotypes. Least growth index were observed in genotypes PBCM1159 (0.46), which is rated as tolerant one, followed by BSH1, NDRC190 and YSRLC-9-26. The digestibility index was maximum (61.2%) on PC5 and minimum (54.4%) on PBCM1159. The larvae digested the leaves of GSL1, GSL2, PGSH51 and PC5 more easily, categorizing these as most susceptible genotypes. PBCM1159 was found to be tolerant based on lowest AD, which came on par with

TABLE 2. Consumption and utilization indices of *P. brassicae* on different genotypes of rapeseed-mustard

Genotype	Consumption index (CI)	Growth rate (GR)	Approximate Digestibility (AD)	Efficiency of conversion of ingested food (ECI)	Efficiency of conversion of digested food (ECD)
<i>Brassica napus</i>					
GSL 1	1.65	0.56	61.5 (51.62)	20.3	43.6 (41.30)
GSL 2	1.90	0.52	58.3 (49.78)	20.0	46.5 (43.00)
PGSH 51	1.87	0.55	59.3 (50.36)	19.8	43.4 (41.18)
PGSH 124	2.24	0.49	56.5 (48.70)	19.2	38.8 (38.50)
<i>B. juncea</i>					
Purple mutant	2.24	0.50	55.9 (48.37)	19.1	42.1 (40.42)
NDRC 190	2.24	0.46	56.6 (48.78)	19.0	35.1 (36.38)
YSRLC 9-26	2.24	0.47	56.5 (48.73)	19.3	39.6 (38.99)
RL 1359	2.34	0.48	56.8 (48.92)	19.3	37.9 (37.97)
PBCM 1159	2.48	0.44	54.4 (47.51)	19.0	36.3 (36.99)
<i>B. campestris</i>					
TL 15	2.28	0.49	56.5 (48.72)	19.0	35.8 (36.74)
BSH 1	2.39	0.46	55.9 (48.36)	19.1	36.0 (36.85)
<i>B. carinata</i>					
PC 5	1.78	0.57	61.2 (51.48)	20.6	44.2 (41.66)
CD ( $p = 0.05$ )	0.10	0.03	(1.67)	NS	(2.64)

Figures in parentheses are *arc sine* transformed values.

rest of the genotypes. The efficiency of conversion of ingested food (ECI) showed only limited variation (19.0 to 20.6 %) and differences among the treatments were not statistically significant. The efficiency of conversion of digested food (ECD) into body substances of *P. brassicae* was higher when fed on GSL2 (46.5%), PC5 (44.2%), GSL1 (43.6%) and PGSH51 (43.4%) and thus these were rated as susceptible. It was lowest on NDRC190 (35.1%), which came on par with PBCM1159 (36.3%), TL15 (35.8%) and BSH1 (36.0%). These genotypes were categorized as comparatively tolerant to *P. brassicae*. Based on CI, Gupta and Maleyvar (1981) rated turnip as more susceptible for *P. brassicae* as compared to radish. Chibber *et al.* (1985) reported that non suitable/tolerant hosts *Solanum melongena*, *Corchorus capsularis* and *Cajanus cajan* adversely affect the growth rate of *Spodoptera litura* than those of suitable hosts. According to Sharma and Lopez (1990), the growth index of sorghum head bug, *Calocoris angustatus* was lower in resistant cultivars as compared to susceptible ones. Lower AD of *P. brassicae* fed on cauliflower, *sarson* and *nasturtium* plants was reported by Kaushal and Vats (1983), which they attributed to the unsuitable nature of these food plants for the pest. On the other hand, cabbage with higher AD was categorized as best host. Das *et al.* (2002) has reported that the lower efficiency of conversion of digested food into body substances by *Heiroglyphus banian*, a pest of paddy, may be due to nutritional deficiency in plant.

The results thus showed that PC 5, GSL 1 and PGSH 51 with low consumption index and higher growth rate, digestibility, and efficiency of conversion of digested food were most susceptible for growth and development of *P. brassicae*. However, PBCM 1159 and BSH 1 with higher consumption index and lower higher growth rate, digestibility, efficiency of conversion of digested food was considered as tolerant for *P. brassicae* and these may be further evaluated for mechanism of resistance.

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## Mosquito biodiversity and distribution in Mandya district, Karnataka State, India

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**ABSTRACT:** Mosquito borne diseases, especially malaria and Japanese encephalitis, are major public health problems in Mandya district of Karnataka state. We studied the species composition, seasonal prevalence, and distribution of mosquitoes in the said district. A total of 143 collection sites covering seven taluks of Mandya district were randomly sampled. The larvae, pupae and adults of different species were collected. The data of both larvae and adults of all collection sites for a particular species were pooled to determine the seasonal prevalence and distribution. Twenty-five species of mosquitoes (both vectors and non-vectors) belonging to five genera, *Anopheles* (13), *Culex* (5), *Aedes* (3), *Mansonia* (3) and *Armigeres* (1) were recorded. The recorded species included vectors of malaria, Bancroftian filariasis, Japanese encephalitis, dengue and dengue haemorrhagic fever. *Anopheles culicifacies*, *An. subpictus*, *An. vagus*, *Culex quinquefasciatus* and *Cx. tritaeniorhynchus* were found in all seasons of the year in all taluks of the district. Other species recorded were sparsely distributed.

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**KEYWORDS:** biodiversity, distribution, Mandya, mosquito, non-vectors, vectors

### INTRODUCTION

District Mandya in Karnataka state is known to be epidemic prone area for malaria and Japanese encephalitis (Mishra *et al.*, 1984; NVBDCP, 2003). Cauvery and its tributaries pass through this district and many streams flow into these rivers. The canal systems existing in majority of the villages are provided with tanks and many of them harbor water weeds. Seepage from the tank bunds provides plenty of marshy areas in villages (Mishra *et al.*, 1984). These conditions provide excellent habitat for mosquito breeding, especially anopheline species, throughout the year. Hence a survey

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was conducted to assess the prevalence and distribution of mosquito fauna in Mandya district.

## MATERIALS AND METHODS

### Study area

One hundred and forty three sites distributed in the seven taluks of the district were randomly selected (Table 1). The immature stages and adults of the mosquitoes were collected once in summer (March–June), rainy (July–October) and winter (November–February) seasons and for a period of three years from July 2002 to June 2005.

### Mosquito collection

Adult mosquitoes were collected from human dwellings and cattle sheds using aspirators and flashlight between 7.00 and 11.00 pm and 4.30 and 7.00 am. Immature stages were collected from different breeding habitats viz. lakes, ponds, pools, tanks, irrigated canals, streams and drainages using enamel tray and dipper. Collections from each site were maintained separately in suitable containers in the insectary. The immature stages were allowed to emerge as adults. The adults were counted and identified using the keys of Christopher (1933), Barraud (1934), Puri (1957) and Nagpal and Sharma (1997). The seasonal prevalence and percent distribution of mosquito species were estimated. The percent distribution of mosquito species was arrived by calculating as: the number of collection sites where specific species occurred  $\times 100$ /the total number of collection sites (143).

In this study, species distributed in 67 to 100% of the collection sites were considered as widely distributed, 33 to 66% as moderately distributed and 1 to 32% as sparsely distributed.

## RESULTS AND DISCUSSION

Altogether 25 species of mosquitoes were recorded in the seven taluks of Mandya District of Karnataka State. The seasonal prevalence of each species in respective taluk is presented in Table 1. Percent distribution of each species is shown in Table 2.

Both vector and non-vector of anopheline and culicine mosquitoes were recorded in the present study. Of the six established malaria vectors in India, only *An. culicifacies* and *An. stephensi* were reported in Mandya district. *An. culicifacies* was found in all seasons and observed in 68 collection sites out of 143 covering 47.55% in Mandya district (Tables 1 and 2). *An. culicifacies* is a well known rural and periurban malaria vector and transmits about 60–70% of total malaria in India (Nagpal and Kalra, 1997). *An. stephensi* was recorded only once in rainy season in the present study (Tables 1 and 2).

*An. annularis* is a secondary vector and was recorded in 24 places during the study period (Table 2) and the same was reported by others in Indonesia, Malaysia, Myanmar, Nepal, Orissa, Bihar and West Bengal (Nagpal and Sharma, 1997). *An.*

TABLE I. Seasonal prevalence of various mosquito species in Mandya district

Sl. No.	Species	Taluk of Mandya District																Srirangapatna			
		KR Per		Maddur		Malavalli		Mandya		Nagamangala		Pandavapura						S	R	S	W
		S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R				
1	<i>An. annularis</i>	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+
2	<i>An. barbirostris</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	<i>An. culicifacies</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	<i>An. janesii</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	<i>An. leyoportensis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	<i>An. maculatus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	<i>An. nigerrimus</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	<i>An. pallidus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	<i>An. philippinensis</i>	-	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+	-	+	+
10	<i>An. stephensi</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	<i>An. subpictus</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	<i>An. tessellatus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	<i>An. vagus</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14	<i>Cx. bitaeniorhynchus</i>	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15	<i>Cx. gelidus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	<i>Cx. mimulus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	<i>Cx. quinquefasciatus</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
18	<i>Cx. tritaeniorhynchus</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
19	<i>Ae. aegypti</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20	<i>Ae. albopictus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	<i>Ae. vittatus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	<i>Mu. annulifera</i>	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
23	<i>Mu. indiana</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	<i>Mu. uniformis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	<i>Ae. subulbatus</i>	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Total species		9	8	14	10	9	12	8	7	15	11	12	10	8	8	7	7	7	7	9	11

S = summer season, R = rainy season, W = winter season. Presence of the species is indicated by plus sign

TABLE 2. Percent distribution of various mosquito species in Mandya district

Species	Percent distribution in each taluk							Total % distributed in Mandya district (143)
	K. R. Pet (24)	Maddur (20)	Malavalli (19)	Mandya (25)	Naganan gala (23)	Pandava pura (16)	Srirangapatna (16)	
<i>An. annularis</i>	8.33(2)	15.00(3)	10.53(2)	28.00(7)	34.78(8)	-	12.50(2)	16.78(24)
<i>An. barbatris</i>	-	5.00(1)	10.53(2)	8.00(2)	-	6.25(1)	-	4.20(6)
<i>An. culicifacies</i>	37.50(9)	25.00(5)	47.37(9)	48.00(12)	86.90(20)	25.00(4)	56.25(9)	47.55(68)
<i>An. jamezii</i>	4.17(1)	5.00(1)	10.53(2)	8.00(2)	-	-	-	4.20(6)
<i>An. jeyporensis</i>	-	-	10.53(2)	-	-	-	-	1.40(2)
<i>An. maculatus</i>	8.33(2)	5.00(1)	-	-	-	-	-	2.10(3)
<i>An. nigerrimus</i>	8.33(2)	20.00(4)	15.79(3)	4.00(1)	4.35(1)	12.50(2)	31.25(5)	12.59(18)
<i>An. pallidus</i>	4.17(1)	-	15.79(3)	4.00(1)	4.53(1)	-	6.25(1)	4.90(7)
<i>An. phuiipinensis</i>	4.17(1)	-	-	-	4.35(1)	-	12.50(2)	2.80(4)
<i>An. stephensi</i>	-	-	-	4.00(1)	-	-	-	0.70(1)
<i>An. subpictus</i>	58.33(14)	85.00(17)	100.00(19)	92.00(23)	100.00(23)	62.50(10)	81.25(13)	83.22(119)
<i>An. tessellatus</i>	-	-	-	-	-	6.25(1)	12.50(2)	2.10(3)
<i>An. vagus</i>	41.67(10)	35.00(7)	57.89(11)	32.00(8)	69.57(16)	43.75(7)	43.75(7)	46.15(66)
<i>Cx. bitaeniorhynchus</i>	12.50(3)	10.00(2)	15.79(3)	20.00(5)	-	6.25(1)	18.75(3)	11.89(17)
<i>Cx. gelidus</i>	-	10.00(2)	10.53(2)	-	-	-	-	2.80(4)
<i>Cx. minimus</i>	4.17(1)	-	-	-	-	-	-	0.70(1)
<i>Cx. quinquefasciatus</i>	62.50(15)	55.00(11)	68.42(13)	84.00(21)	69.57(16)	62.50(10)	81.25(13)	69.23(99)
<i>Cx. tritaeniorhynchus</i>	29.17(7)	60.00(12)	73.68(14)	52.00(13)	43.48(10)	68.75(11)	81.25(13)	55.94(80)
<i>Ae. aegypti</i>	33.33(8)	20.00(4)	5.26(1)	12.00(3)	17.39(4)	12.5(2)	18.75(3)	17.48(25)
<i>Ae. albopictus</i>	4.17(1)	10.00(2)	-	-	-	-	-	2.10(3)
<i>Ae. vittatus</i>	-	5.00(1)	10.53(2)	-	-	-	6.25(1)	2.80(4)
<i>Ma. annulifera</i>	-	-	-	4.00(1)	-	-	-	0.70(1)
<i>Ma. idiana</i>	-	-	-	4.00(1)	-	-	-	0.70(1)
<i>Ma. uniformis</i>	-	5.00(1)	-	-	-	-	-	0.70(1)
<i>Ar. sulabatus</i>	-	20.00(4)	36.84(7)	4.00(1)	17.39(4)	-	6.25(1)	11.89(1)

Figures in parentheses show the number of collection sites where the species occur.

*jeyporiensis* and *An. philippinensis* reported in the present study are also secondary vectors in India, China, Indo-China and Myanmar (Nagpal and Sharma, 1997).

*An. barbirostris* was sparsely distributed in Mandya district (Table 2). This species is a vector for human filariasis in India and Indonesia and also a vector for malaria in Indonesia (Wattal, 1961; Horsfall, 1972). *An. nigerrimus* was recorded in 18 places (Table 2). The said species is a known malaria vector in Indonesia and Malaysia and vector for human filariasis in India, Malaysia, Thailand and Sri Lanka (Nagpal and Sharma, 1997). The distribution of *An. maculatus* was sparse (Table 2) and is an important vector of malaria in Malaysia and also a suspected vector in North Eastern States of India (Nagpal and Sharma, 1997). *An. subpictus* was found in all seasons and widely distributed occurring in 119 sites in the present study (Table 2). This species is a human filariasis vector (Raghavan, 1969) and also suspected vector for malaria in India (Panicker *et al.*, 1981) and main malaria vector in Sri Lanka (Nagpal and Sharma, 1997). *An. tessellatus* was sparsely distributed in the district (Table 2). This species is also considered as a suspected vector in Lakshadweep Islands of India (Nagpal and Sharma, 1997). *An. jamesii*, *An. pallidus* and *An. vagus* are considered as non-vectors (Nagpal and Sharma, 1997). *An. jamesii* and *An. pallidus* were found sparsely distributed and *An. vagus* was seen in all seasons and widely distributed in Mandya district (Tables 1 and 2).

Among the culicine mosquito species recorded in the study, *Cx. quinquefasciatus* is vector for Bancroftian filariasis; *Cx. tritaeniorhynchus*, *Cx. gelidus*, *Ma. annulifera*, *Ma. indiana* and *Ma. uniformis* are vectors for Japanese encephalitis (Reuben and Gajanana, 1997). *Ae. aegypti* is vector for dengue, dengue hemorrhage fever and yellow fever *Ae. albopictus* is secondary vector for dengue, and dengue hemorrhage fever whereas *Cx. bitaeniorhynchus*, *Cx. mimulus*, *Ae. vittatus* and *Ar. subalbatus* are considered as non-vectors. *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus* were found in all seasons and widely distributed in the Mandya district. Rest of the culicine species were sparsely distributed (Tables 1 and 2).

For the first time, extensive studies on biodiversity of mosquitoes in the Mandya district of Karnataka state (India) have been undertaken. The knowledge of the presence of mosquito species at a particular time can help to predict the possibility of disease spread by mosquito species and this could help to take preventive measures well in advance. Such systematic studies go a long way in establishing the mosquito species distribution and also the distribution of mosquito vectors in different parts of the country. This would also enable us to prepare a mosquito atlas of India.

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## Alterations in qualitative and quantitative traits in the bivoltine silkworm hybrids of *Bombyx mori* L. due to thermal stress

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**ABSTRACT:** Seven authorized bivoltine silkworm hybrids viz., CSR2 × CSR4, CSR2 × CSR5, CSR3 × CSR6, CSR12 × CSR6, CSR16 × CSR17, CSR18 × CSR19 and CSR50 × CSR51 were reared under temperatures of  $25 \pm 1^\circ\text{C}$ ,  $28 \pm 1^\circ\text{C}$  and  $32 \pm 1^\circ\text{C}$  with constant RH of  $75 \pm 5\%$ . Drastic reduction in qualitative and quantitative characters was recorded when the larvae were exposed to high temperature ( $32 \pm 1^\circ\text{C}$ ). Among the hybrids, CSR18 × CSR19 and CSR50 × CSR51 revealed more tolerance to high temperature as indicated by high survival and cocoon yield. © 2007 Association for Advancement of Entomology

**KEYWORDS:** *Bombyx mori* L., bivoltine silkworm hybrids, thermal stress

### INTRODUCTION

In tropical countries sericulture has received a big boost because of the increasing demand for silk world over. Many under developed and developing countries concentrate on sericulture since it helps in poverty alleviation. In many countries in this belt the climatic factors are not fully favourable for this industry. Among the environmental stress components, temperature regime is the most important. Efforts are being made for developing bivoltine breeds/hybrids with a view to increase the yield. Therefore, realizing the need for high cocoon shell percentage and high raw silk percentage as thrust areas many viable bivoltine single hybrids have been evolved and authorized for commercial exploitation (Basavaraja *et al.*, 1995; Datta *et al.*, 2001; Dandin *et al.*, 2006). In this context the effect of different temperature regimes on qualitative and quantitative characters of the bivoltine silkworm hybrids was assessed.

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## MATERIALS AND METHODS

Seven authorized bivoltine silkworm hybrids viz., CSR2 × CSR4, CSR2 × CSR5, CSR3 × CSR6, CSR12 × CSR6, CSR16 × CSR17, CSR18 × CSR19 and CSR50 × CSR51 were utilized in the present study. Three composite layings of 500 eggs each taken from 10 disease free layings were used for each hybrid. After third moult, 300 larvae were retained in each replication and they were subjected to thermal stress. Rearing was conducted at  $25 \pm 1^\circ\text{C}$ ,  $28 \pm 1^\circ\text{C}$  and  $32 \pm 1^\circ\text{C}$  under RH of  $75 \pm 5\%$ . Ten cocoons each of female and male were used for assessment of cocoon weight, shell weight and shell percentage. Fifty reelable cocoons from each replication were used for test reeling to assess the post cocoon parameters such as filament length, raw silk percentage, filament size (denier) and reelability. The data were statistically analysed.

## RESULTS AND DISCUSSION

The rearing performance of hybrids at different temperatures is given in Table 1. Drastic reduction ( $P > 0.05$ ) in majority of characters was recorded when the larvae were reared at  $32^\circ\text{C}$ . The most affected characters are cocoon yield followed by shell weight, cocoon weight, survival, reelability, filament length and raw silk percentage. Least affected characters are shell percentage and filament size. Marginal reduction in cocoon yield, shell weight and filament length was also recorded when the larvae were exposed to  $28^\circ\text{C}$ . Significant reduction in survival occurred when the larvae were exposed to  $32^\circ\text{C}$ . At this temperature the hybrids CSR18 × CSR19 and CSR50 × CSR51 recorded higher survival (86.0% and 85.4%, respectively) than other hybrids. The cocoon yield, cocoon weight and shell weight were significantly altered among the hybrids reared both at  $28^\circ\text{C}$  and  $32^\circ\text{C}$ . The hybrid CSR50 × CSR51 recorded better cocoon yield (14.4 kg) followed by CSR16 × CSR17 (13.0 kg) reared at high temperature ( $32^\circ\text{C}$ ). The character shell percentage is not affected significantly in all hybrids reared at temperature of  $32^\circ\text{C}$ . This may be attributed to the hybrid characteristic, which is determined genetically. Significant reduction in reeling parameters viz., filament length and raw silk % was recorded in all hybrids reared both at  $28^\circ\text{C}$  and  $32^\circ\text{C}$ . The wide variations recorded among hybrids for majority of characters particularly at high temperature ( $32^\circ\text{C}$ ) indicate that these hybrids differ in their genetic make up and thereby variation in tolerance capacity to high temperature. The hybrids, CSR18 × CSR19 and CSR50 × CSR51 have shown less reduction for characters like survival and cocoon yield at high temperature ( $32^\circ\text{C}$ ) indicating more tolerance to temperature than other hybrids.

The low survival on exposure of larvae to high temperature levels could be attributed to the low feeding activity of the silkworm which results in physiological derangement and poor health of the larvae and thereby decreased cocoon characters (Ueda and Lizuka, 1962; Takeuchi *et al.*, 1964). The phenomenon of cocoon yield is correlated to cocoon weight and because of high temperature stress, the cocoon weight is drastically reduced affecting the cocoon yield.

TABLE 1. Effect of temperature stress on qualitative and quantitative characters in bivoltine silkworm hybrids

Hybrids tested	Survival (%)			Cocoon yield/10,000 larvae (kg)			Cocoon weight (g)		
	25 ± 1°C	28 ± 1°C	32 ± 1°C	25 ± 1°C	28 ± 1°C	32 ± 1°C	25 ± 1°C	28 ± 1°C	32 ± 1°C
CSR2 × CSR4	91.5	91.5	66.0	20.7	18.0	11.2	2.10	1.97	1.69
CSR2 × CSR5	90.8	90.8	63.0	20.9	18.8	10.7	2.21	2.07	1.70
CSR3 × CSR6	91.3	91.3	69.0	20.7	18.5	11.5	2.17	2.03	1.67
CSR12 × CSR6	93.2	93.2	72.4	20.9	18.5	9.1	2.11	1.98	1.25
CSR16 × CSR17	93.5	93.5	77.0	21.3	19.0	13.0	2.15	2.03	1.75
CSR18 × CSR19	95.0	95.0	86.0	18.5	16.9	11.3	1.90	1.78	1.32
CSR50 × CSR51	94.5	94.5	85.4	20.9	18.8	14.4	2.16	1.99	1.69
CD at 5%	NS	NS	3.6	1.1	0.8	1.0	0.13	0.09	0.09
Hybrids × Temperature		4.4			1.0			0.10	
Hybrids tested	Shell weight (cg)			Shell percentage (%)			Filament length (m)		
	25 ± 1°C	28 ± 1°C	32 ± 1°C	25 ± 1°C	28 ± 1°C	32 ± 1°C	25 ± 1°C	28 ± 1°C	32 ± 1°C
CSR2 × CSR4	49.8	46.1	38.4	23.7	23.4	22.7	1160	1082	868
CSR2 × CSR5	53.2	48.0	38.9	24.0	23.2	22.9	1206	1077	867
CSR3 × CSR6	52.1	48.3	39.5	24.1	23.8	23.7	1274	1086	970
CSR12 × CSR6	51.4	46.5	27.6	24.4	23.5	22.1	1274	1116	938
CSR16 × CSR17	49.7	46.5	39.9	23.2	22.9	22.8	1151	1026	960
CSR18 × CSR19	39.6	35.6	28.7	20.8	20.0	20.8	1068	946	807
CSR50 × CSR51	52.3	47.4	39.7	24.2	23.8	23.5	1250	1050	925
CD at 5%	2.7	3.1	3.3	0.8	1.3	1.1	46	87	30
Hybrids × Temperature		2.9			0.9			64	
Hybrids tested	Raw silk %			Reelability (%)			Filament size (d)		
	25 ± 1°C	28 ± 1°C	32 ± 1°C	25 ± 1°C	28 ± 1°C	32 ± 1°C	25 ± 1°C	28 ± 1°C	32 ± 1°C
CSR2 × CSR4	19.7	18.6	15.6	84.7	85.3	72.5	3.02	2.79	2.56
CSR2 × CSR5	19.8	18.1	15.4	87.0	83.0	73.0	3.15	2.66	2.50
CSR3 × CSR6	20.0	19.2	16.9	88.0	85.3	68.5	2.75	2.84	2.56
CSR12 × CSR6	20.3	18.0	14.4	85.7	84.0	65.0	3.00	2.85	2.45
CSR16 × CSR17	19.6	19.3	15.9	87.3	88.7	66.0	2.75	2.54	2.52
CSR18 × CSR19	17.9	17.8	15.3	86.0	85.0	74.0	2.80	2.56	2.37
CSR50 × CSR51	19.8	18.6	16.3	87.3	84.7	74.0	2.98	2.80	2.60
CD at 5%	0.3	1.1	0.7	NS	2.3	3.36	NS	0.2	NS
Hybrids × Temperature		0.8			2.4			0.3	

NS. non significant

The hot climatic conditions prevailing particularly in summer are not conducive to rear the high yielding bivoltine hybrids throughout the year. Considering the poor performance of bivoltine hybrids during this season, emphasis was given to develop races suitable for high temperature. This has led to the development of compatible bivoltine hybrids for rearing throughout the year by utilizing Japanese hybrids as breeding resource material (Datta *et al.*, 2001) and it was suggested that any study involving temperature as one of the environmental factors affecting the viability followed by cocoon traits is a trend setter to provide basic knowledge to formulate

appropriate strategies for selecting the breeds/hybrids for adverse climatic conditions of tropics.

Among the many factors that are attributed to the poor performance of bivoltine strains under tropical conditions, the most important aspect is that many quantitative characters such as survival and cocoon traits decline sharply when temperature increases over 28 °C. The low adaptability of bivoltine breeds/hybrids to these tropical environment conditions make them unsuitable for commercial exploitation throughout the year. The silkworm breeds/hybrids reared over a series of environments exhibiting less variation are considered more stable. Therefore, the hybrids CSR2 × CSR4, CSR2 × CSR5, CSR3 × CSR6, CSR12 × CSR6 and CSR16 × CSR17 are recommended only for rearing during favourable months where the temperature is ideal for silkworm rearing. The hybrids CSR18 × CSR19 and CSR50 × CSR51 which have shown less reduction for characters like survival and cocoon yield than other hybrids can be reared throughout the year.

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## Resistance of wild and cultivated sesame (*Sesamum* spp.) to *Antigastra catalaunalis* (Dup.) (Lepidoptera: Pyralidae)

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**ABSTRACT:** *Sesamum indicum* L. and its wild relatives (*S. alatum* Thonn., *S. laciniatum* Klein, *S. mulayanum* Nair, *S. malabaricum* Burm., *S. radiatum* Schumacher & Thonn) were evaluated in the laboratory for resistance to *Antigastra catalaunalis* (Dup.) On cultivated sesame (*S. indicum*), the development period of *A. catalaunalis* was longer than on wild species. Larval survival ranged from 10.25% to 35% on wild species while it was 90% on cultivated species. Growth indices and survival index were highest for larvae reared on *S. indicum*. Low damage was caused to seeds of wild species when given for feeding under laboratory conditions. The study has identified a source of *A. catalaunalis* resistance in wild *Sesamum* spp.

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**KEYWORDS:** plant resistance, biology, *A. catalaunalis*, sesame, wild plant relatives

### INTRODUCTION

Sesame, *Sesamum indicum* L. is known to be infested by 65 species of insects and a mite (Ahuja and Bakhetia, 1995). Among these, the leaf webber/capsule borer, *Antigastra catalaunalis* (Dup) is reported to be a major constraint in raising the productivity of sesame. Protection through insecticides has not been found remunerative under rain fed farming situation (Ahuja, 1999). Providing protection in seed itself by transferring resistant genes through crossbreeding with resistant genotypes may provide suitable alternative to insecticides. It may also reduce the application of insecticides and make pest control programme ecofriendly. Very few confirmed sources of resistance to *Antigastra catalaunalis* in cultivated species of

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sesame have been reported (Ahuja and Kalyan, 2001). The present studies were conducted with wild cultivars of sesame to look for alternative sources of resistance in sesame against *A. catalaunalis*.

#### MATERIALS AND METHODS

The advanced stage *A. catalaunalis* larvae (third and fourth instar) were collected from the farmers' and research fields of Agricultural Research Station, Mandor, Jodhpur to raise a nucleus culture in the laboratory as per method described by Ahuja *et al.* (2002). The insect was reared on TC 25 variety of *S. indicum*. Fresh twigs were kept with the base immersed in water kept in tubes (7.5 cm × 5 cm) wound with cotton round the stem at the opening of the tube. The tubes were then placed inside plastic cages, 37.5 × 30 cm. Four holes, 12.5 cm × 12.5 cm, covered with wire mesh provided aeration. Wet sand was placed at bottom to maintain humidity. Cages were covered with wet muslin cloth. For conducting biology studies, first instar larvae (0–24 h) were transferred on test host plants. The cages were kept in BOD incubator at  $27 \pm 0.5^\circ\text{C}$  and 75% RH under 12 h light and 12 h dark periods. Food was changed daily. Observations were recorded on larval period, pupal period, number of larvae pupated and number of adults emerged. Five replications were maintained for the experiment. Various growth indices as suggested by Howe (1971) and Srivastava (1959) and survival index (Ahuja and Sehgal, 1982) were computed. The biology of the pest was studied on five wild species of sesame namely *S. alatum* Thonn., *S. laciniatum* Klein., *S. mulayanum* Nair, *S. malabaricum* Burm and *S. radiatum* Schumacher & Thonn procured from regional station of National Bureau of Plant Genetics Resources, Akola, Maharashtra, in comparison with T.C. 25 variety of *S. indicum*. In a field experiment the above varieties were sown in 5 meter row length at the onset of monsoon along with agronomically suitable variety (T.C. 25) of *S. indicum* at research farm of Agricultural Research Station, Mandor, Jodhpur. At the pod formation stage, green pods were plucked, cut open and fed to fourth instar larvae and percent seed damage was worked out (Baskaran *et al.*, 1990).

#### RESULTS AND DISCUSSION

The larval period was similar in all the wild species and ranged from 9.33–9.75 days which was significantly lower than the period in *S. indicum* (12.55 days) (Table 1). But pupal period on the wild species was a little higher than on the cultivated species. On wild species, time taken to complete the development from larval stage to adult stage ranged from 15.5 to 16 days; the corresponding duration on cultivated species was 17.62 days that was significantly higher. Larval survival on wild species varied from 10.25 per cent on *S. malabaricum* to 35 per cent on *S. alatum*. No adult emergence was recorded on *S. malabaricum* and it ranged from 5.55 to 11.76 per cent on other wild species of sesame; the corresponding value for *S. indicum* was 80 per cent.

Growth and development of *A. catalaunalis* was adversely affected on wild species of sesame. This induced early pupation resulting in lower larval period, and high larval

TABLE 1. Development of *Antigastra catalaunalis* on cultivated and wild species of sesame and damage caused to seeds under laboratory conditions

Host Plant	Larval period (days)	Pupal period (days)	Combined larval pupal period (days)	Pupation (%)	Adult emergence <sup>1</sup> (%)	Survival index	Growth index (N/A)	Howe's growth Index (Log N/Av)	Seed damage <sup>2</sup> (%)
Cultivated species of sesame									
<i>S. indicum</i>	12.55	5.06	17.62	90.00	80.00	1.00	4.54	0.11	22.67
Wild species of sesame									
<i>S. alatum</i>	9.42	5.50	15.50	35.00	10.00	0.12	0.65	0.065	0.0
<i>S. laciniatum</i>	9.40	6.00	16.00	25.00	11.11	0.13	0.69	0.065	0.0
<i>S. mulayanum</i>	9.50	5.50	16.00	30.00	11.11	0.13	0.69	0.061	0.0
<i>S. malabaricum</i>	9.33	—	—	10.25	—	—	—	—	2.0
<i>S. radiatum</i>	9.75	6.00	16.00	20.00	5.55	0.06	0.34	0.048	3.0
CD	0.74	0.39	00.74	14.63	16.38				

<sup>1</sup> Calculated on the basis of larvae released initially. <sup>2</sup> Percentage of seed damaged by fourth instar larvae under laboratory conditions.

and pupal mortality. Among wild species, *S. malabaricum* did not support the growth and development of *A. catalaunalis*. Non-suitability of wild species as host plant was also evident from the lower damage caused to seeds (0–3.0%) when given for feeding under laboratory conditions (Table 1). No damage was recorded on any portion of the plant of wild species under field conditions. Negligible leaf and seed damage by the larvae of *A. catalaunalis* was reported in *S. alatum* under field conditions earlier (Baskaran *et al.*, 1990). The growth indices were higher on cultivated species as compared to wild species. The data suggested that wild species were not suitable for growth and development of *A. catalaunalis*. No studies are available on the biology of *A. catalaunalis* on wild species of sesame; however, resistance to *A. catalaunalis* in allotetraploid formed as a result of cross between *S. indicum* and *S. mulayanum* (Biswas and Bose, 1998) and F<sub>1</sub> hybrids between *S. indicum* cv. WB 67 (female) and wild *S. mulayanum* (Biswas and Mitra, 1990) has been reported. There is scope for developing *A. catalaunalis* resistant varieties against the pest in cultivated species of sesame through interspecific hybridization using wild species identified in this experiment.

#### ACKNOWLEDGEMENTS

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## Description of a new species of mite *Pronematus oryzae* (Prostigmata: Tydeidae) from India

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**ABSTRACT:** A new species of predatory mite, *Pronematus oryzae* (Prostigmata: Tydeidae), collected from leaf sheaths of rice at Delhi, India is described. This mite was observed to be predatory on the phytophagous *Oligonychus indicus* (Hirst) (Tetranychidae). It is distinguished from other species of the genus in having tarsus I longer than tibia I; distal tarsal setae serrate till the tip; solenidion median, small in female, longer than tarsus I in male, slightly bent, broader at base and stout; sensory setae at least 2x the length of propodosomal setae P<sub>1</sub> and P<sub>2</sub>; all dorsal setae serrate till tip and ventral setae almost 0.33x the distance between their bases.

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**KEYWORDS:** *Pronematus oryzae* sp. nov., Tydeidae, predator, *Oligonychus indicus*, rice

### INTRODUCTION

*Pronematus* is a widespread but little-known genus. These mites are vagrant in their habitats, observed feeding on honey dew, lichen, moss, fungi, dead insects, other mite or mite fragments and inhabiting nests of birds. Several *Pronematus* spp. have been reported from different parts of India. Gupta and Dhooria (1972) observed *P. elongatus* Baker in association with vineyard mite *Eriophyes vitis* (Pagenstecher). Pandey *et al.* (1979) reported *Pronematus* sp. near *anconai* Baker from *Calotropis procera* (Aiton) and *P. mcgregori* Baker from *Clerodendron inerme* (L.). Gupta and Paul described *P. bengalensis* and *P. indiana* (1985), and *P. saularis* (1992), all from bird's nests. Mukherjee and Singh (1993) reported *P. fleschneri* Baker, *P. ubiquitous* (McGregor) and *P. sextoni* Baker from various fruit trees. Tagore and Putatunda (2003) reported

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*P. fleschneri* infesting *Tecoma stans* (L.). Several unidentified species of *Pronematus* have also been reported from house dust samples (Modak *et al.*, 1992; Saha and Modak, 1993), vegetables (Kapur-Ghai and Bhullar, 2003) and from stored food products (Putatunda, 2005).

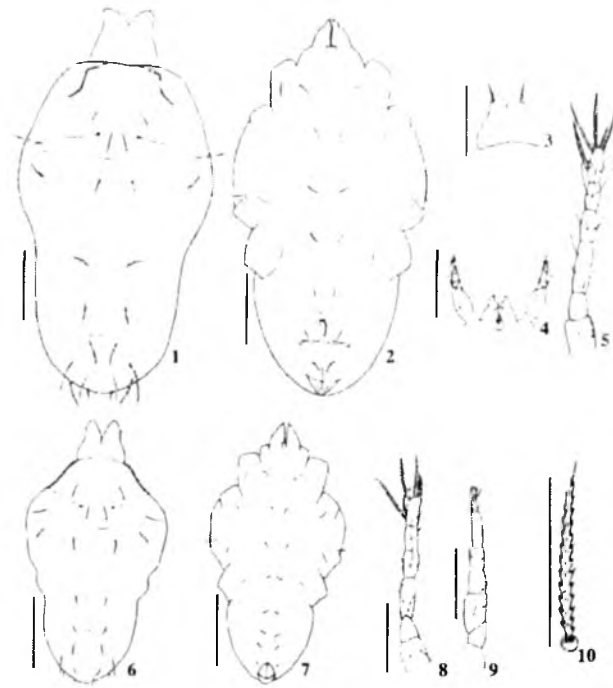
The present paper describes a new species of *Pronematus*, collected from rice fields along with other mites associated with rice agroecosystems at Delhi namely, *Lasioseius terrestris* Menon & Ghai (Ascidae); *Amblyseius imbricatus* Corpuz & Rimando, *Amblyseius alstoniae* Gupta, *Amblyseius delhiensis* (Narayanan & Kaur), *Amblyseius ovalis* (Evans) (Phytoseiidae); *Steneotarsonemus spinky* Smiley (Tarsonemidae) and *Oligonychus indicus* (Hirst) (Tetranychidae). The genus identification and terminology used is based on Baker (1968). All measurements indicated are mean values; length of the body is the distance from the tip of the rostrum to the posterior end of the body in dorsal view while width of the body is the broadest region of the podosoma.

### Genus *Pronematus* Canestrini 1886

*Pronematus oryzae* sp. nov. (Figs. 1–10)

**Female** ( $n = 40$ ): Length  $268 \pm 36.25 \mu$ ; width  $131 \pm 19.3 \mu$  (Figs. 1, 2). Body colour pale yellow to colourless; rostrum long and broad, strongly cleft anteriorly; movable chelae strong and typical, needle like (Fig. 3); distal palpal segment with rough protuberances, at least 0.5x longer than penultimate segment, which is slightly broader (Fig. 4). Propodosomal striae indistinct; sensory setae  $29.4 \pm 3.13 \mu$ , slightly serrate, more than 2x as long as propodosomal setae  $P_1 12.6 \pm 2.23 \mu$  and  $P_2 10.3 \pm 2.39 \mu$  but almost 2x that of  $P_3 16.8 \pm 3.72 \mu$ ;  $P_1$  present inside and slightly posterior to sensory setae,  $P_2$  pilose and slender as compared to  $P_1$  and  $P_3$ , which are stout and serrate till tip,  $P_3$  longest. Hysterosomal striae indistinct; all dorsal setae stout and serrate till tip (Fig. 10); posterior dorsal setae longer than others; lateral seta  $L_3$  longest.  $D_1 11.3 \pm 2.26 \mu$ ,  $D_2 11.6 \pm 1.84 \mu$ ,  $L_1 12.3 \pm 2.7 \mu$ ,  $L_2 13.2 \pm 2.65 \mu$ ,  $D_3 16.8 \pm 3.72 \mu$ ,  $D_4 19.9 \pm 4.7 \mu$ ,  $D_5 11.1 \pm 3.53 \mu$ ,  $L_3 27.7 \pm 5.32 \mu$  and  $L_4 18.2 \pm 4.08 \mu$ . Ventral body setae short,  $9 \pm 1.3 \mu$ , slender, pilose, length almost 0.33x as the distance between their bases. Tarsus I  $27.1 \pm 3.38 \mu$ , slightly longer than tibia I  $20.7 \pm 2.71 \mu$ ; distal tarsal setae serrate till tip and of varying length, one pair longer, one seta equal and one seta shorter than tarsus I. Solenidion median, small and simple. Leg setae other than those on tarsus I slender, distinct with few serrations (Fig. 5). Length of tarsus II, III and IV at least 0.5x as long as the respective tibia and bears pad like empodium with a pair of claws. Setae of other segments of legs II, III and IV distinct with few serrations.

**Male** ( $n = 10$ ): Smaller than female. Length  $201 \pm 9.7 \mu$ ; width  $97 \pm 3.3 \mu$  (Figs. 6, 7); sensory setae  $28 \pm 1.7 \mu$ ; propodosomal setae  $P_1 12 \pm 1.2 \mu$ ,  $P_2 9.3 \pm 1.2 \mu$  and  $P_3 15 \pm 1.4 \mu$ . Dorsal body setae serrate.  $D_1 9 \pm 1.3 \mu$ ,  $D_2 8.5 \pm 1.3 \mu$ ,  $L_1 9.5 \pm 1.1 \mu$ ,  $L_2 8.5 \pm 1.3 \mu$ ,  $D_3 8.8 \pm 1.3 \mu$ ,  $D_4 7.8 \pm 0.8 \mu$ ,  $D_5 5 \pm 0.1 \mu$ ,  $L_3 13 \pm 2.3 \mu$ , and  $L_4 8.3 \pm 1.2 \mu$ . Tarsus I  $26 \pm 1.6 \mu$ ; tibia I  $20 \pm 1.1 \mu$ . Solenidion median, longer than tarsus I; bent, broader at base and stout (Fig. 8). Dorsal spur on femur IV present (Fig. 9).



FIGURES 1–10. 1. *Pronematus oryzae* sp. nov. 1. ♀ Dorsal view, 2. ♀ Ventral view, 3. ♀ Chelicerae, 4. ♀ Pedipalp, 5. ♀ Leg I, 6. ♂ Dorsal view, 7. ♂ Ventral view, 8. ♂ Leg I with solenidion on tarsus, 9. ♂ Leg IV with dorsal spur on femur, 10. Dorsal seta enlarged. Scale: Figs. 1–9, 50  $\mu$ . Fig. 10, 20  $\mu$ .

### Material

**Holotype:** 1♀, ex *Oryza sativa*, IARI fields, New Delhi, 4.X.06, Pratibha Menon Coll; **Paratype:** 40♀♀ and 10♂♂. Same data as above but different dates: 01.VIII.06 (2♀♀, 1♂), 21.VIII.06 (1♀), 23.VIII.06 (3♀♀), 28.VIII.06 (6♀♀, 1♂), 06.IX.06 (4♀♀), 15.IX.06 (1♂), 16.IX.06 oooooooooo (1♀♀), 21.IX.06 (5♀♀, 1♂). 26.IX.06 (1♀, 2♂♂), 28.IX.06 (8♀♀, 4♂♂), 29.IX.06 (1♀), 30.IX.06 (3♀♀) and 04.X.06 (5♀♀); deposited in National Pusa Collection, Indian Agricultural Research Institute, New Delhi.

### Host

*Pronematus oryzae* was collected from leaf sheaths of rice, found in association with the phytophagous *Oligonychus indicus* exhibiting a predator–prey relationship. Several reports of *Pronematus* as predators on various phytophagous mites are available from India (Gupta *et al.*, 1971; Sadana and Kanta, 1971; Gupta, 1975; Putatunda *et al.*, 1975; Dhooria, 1982; Kapoor *et al.*, 1997).

### Distribution

India: Delhi

### Etymology

Derived from the generic name of the type host plant *Oryza*.

### Comments

*Pronematus oryzae* differs from *P. mcgregori*, *P. biminiensis* Baker, *P. curtitarsus* Baker, *P. bachewingi* Baker, *P. tenuisetosus* Meyer & Rodrigues, *P. davisi* Baker, *P. bengalensis*, *P. indiana* and *P. saularis* in having its tarsus I longer than tibia I; differs from *P. anconai*, *P. elongatus* and *P. neoelongatus* Baker in having distal setae of tarsus I serrate along its entire length; differs from *P. ubiquitus* and *P. rykei* Meyer & Rodrigues in having length of ventral body setae almost 0.33x the distance between their bases; differs from *P. fleschneri* in having a median solenidion on tarsus I; differs from *P. sextoni* in having sensory setae at least 2x longer than propodosomal setae  $P_1$  and  $P_2$ ; differs from *P. curtipilus* Baker in having at least a pair of its distal tarsal setae longer than tarsus I; differs from *P. leucohippeus* Treat in the relative length of dorsal body setae and from *P. staerki* (Schruft) in not having setae on trochanter I.

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## **Involvement of acid phosphatase in degeneration of the silk gland of the tasar silkworm, *Antheraea mylitta* (Drury) (Lepidoptera: Saturniidae)**

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**ABSTRACT:** Increased level of phosphatase activity during the period of degeneration of the silk gland of the tasar silkworm, *Antheraea mylitta* was demonstrated.

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**KEYWORDS:** silk gland, acid phosphatase, *Antheraea mylitta*

Histological studies revealed degeneration of silk gland in the last instar larva of *Antheraea mylitta* (Lepidoptera: Saturniidae) during 18 to 21-day period (Barsagade and Tembhare, 2000). Electron microscopic studies showed a large number of lysosomes attacking nuclear and cytoplasmic organelles and causing degeneration of the gland (Ghonmode and Tembhare, 2003). Degeneration of the silk gland in *A. mylitta* was also found to be due to the effect of  $\beta$ -ecdysone (Tembhare and Ghonmode, 2002). The present study was therefore undertaken to investigate involvement of acid phosphatase in degeneration of silk gland in *A. mylitta*.

From the culture of *A. mylitta* maintained in the Central Tasar Research and Training Institute (CTRTI), Basic Seed Multiplication and Training Centre (BSMTC), Dawadipar, Bhandara (M.S.), India, the last instar larvae were sacrificed on 3, 6, 9, 12, 15, 18, 21 and 24 days of their emergence. The silk glands were dissected and processed for estimation of the acid phosphate activity using the modified method of King and Armstrong (Gutman and Gutman, 1940).

The total acid phosphatase activity increased rapidly in the silk gland of *A. mylitta* in 18–21 day old larvae (regression phase) and thereafter, dropped in 21–24 day old larvae (degeneration phase) (Fig. 1).

Our histological and electron microscopic studies suggested the period of 18 to 21 days as a regression phase of the silk gland in the larvae of *A. mylitta* (Ghonmode and Tembhare, 2003). Involvement of acid phosphatase in degeneration of silk gland in the

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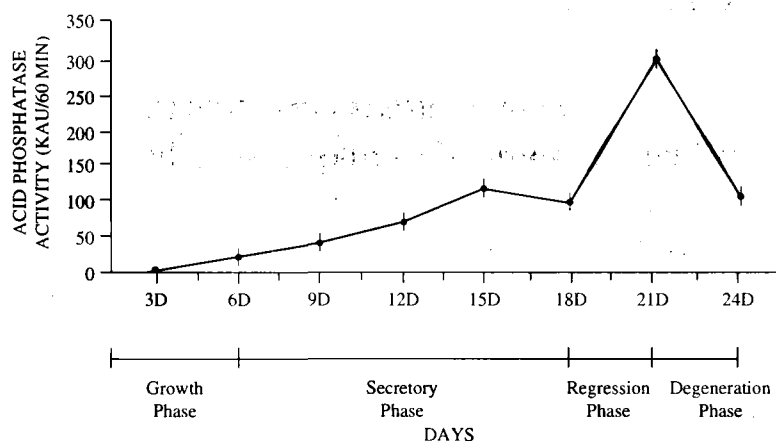


FIGURE 1. Total acid phosphatase activity in the silk gland of last instar larvae of *Antheraea mylitta*

silkworms *Bombyx mori* and *Galleria mellonella* has earlier been reported (Sehna and Michalik, 1984). This report confirms the role of acid phosphatase in degeneration of silk gland in *A. mylitta*.

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## Biology of *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) on some of its major weed hosts

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**ABSTRACT:** Development of *Spodoptera litura* on weed hosts was studied. Out of 24 weed plants tested, high consumption of leaves was recorded on eight species. Further studies were conducted on the development of *S. litura* on these highly preferred weeds, namely *Alternanthera philoxeroides* Mart., *Euphorbia hirta* L., *Eichhornia crassipes* Mart., *Trianthema portulacastrum*, *Parthenium hysterophorus* L., *Cichorium intybus* L., *Rumex obtusifolius* L. and *Ipomoea fistulosa* Mart. There was significant difference in the developmental of the insect on different host plants. Among all the weeds, *T. portulacastrum* was found to be the most suitable food plant. Fast development of *S. litura* on many weed hosts indicates the high potential of the insect as a pest in the agrosystem. © 2007 Association for Advancement of Entomology

**KEYWORDS:** fecundity, oviposition, *Spodoptera litura*, weed hosts

The growth, development and host range of *Spodoptera litura* has been extensively studied on crop plants (Sharma, 1994; Patel *et al.*, 1987; Khuhro *et al.*, 1986; Garad *et al.*, 1985; Balasubramaniam *et al.*, 1984). The species is known to feed, thrive and reproduce on various weeds occurring in and around cultivated field but its biology on weed hosts has been little studied. Therefore, effect of host weeds on the reproductive performance and development of *S. litura* was studied to assess the role of these weeds in the population levels of *S. litura* in an agro-ecosystem.

A culture of *S. litura* was maintained on *Trianthema portulacastrum* L. leaves in the laboratory. Ten day old larvae were used for assessing the feeding preference on 24 species of weeds collected from field. Ten larvae were placed on a bouquet of each plant species kept in well aerated containers. Three replicates were maintained for each plant. Extent of leaf consumption was visually assessed and those showing high consumption of the weed were chosen for further studies. Eight such hosts were identified. For assessing the biological parameters, moths collected from each host were separately confined in suitable containers for egg laying and different developmental parameters were monitored. Survival percentages of larvae and pupae on different host were also assessed. In all tests, replicate results were subjected to

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analysis of variance using Genstat. Treatment means were compared with the least significant difference (LSD) at  $P = 0.05$ .

Among the 24 weeds tested, adequate feeding was observed on eight viz., *Alternanthera philoxeroides* Mart., *Euphorbia hirta* L., *Eichhornia crassipes* Mart., *T. portulacastrum*, *Parthenium hysterophorus* L., *Cichorium intybus* L., *Rumex obtusifolius* L. and *Ipomoea fistulosa* Mart. and they were selected for studying the development details of *S. litura*.

Development on different host plants varied (Table 1). Lowest incubation period of eggs was observed on *T. portulacastrum* and *C. intybus* (2.3 d) while maximum incubation period of eggs was seen on *E. hirta* leaves (5 d).

Minimum and maximum larval period of 16.6 and 32.2 days was noticed in larvae fed on *T. portulacastrum* and *P. hysterophorus* leaves, respectively. Jamil *et al.* (1984) observed larval period of 20 days on *E. crassipes* while we observed 27 days. Percentage survival of larvae was maximum on *T. portulacastrum* (93.3%) and minimum on *P. hysterophorus* (46.7%). Larval weight was also significantly influenced by different hosts. Maximum larval weight (as on 15th day) was observed on larvae fed on *R. obtusifolius* (1.1 g) while minimum larval weight (0.1 g) was recorded on both *E. hirta* and *P. hysterophorus*.

Maximum pupal period was observed in larvae fed on *E. hirta* (16.4 d) and minimum in those fed on *R. obtusifolius* (10 d). Pupal weight was highest on *T. portulacastrum* (0.34 g) and lowest on *E. crassipes* (0.21 g). Percentage survival of the pupa was maximum on *T. portulacastrum* (86.7%) and minimum on *P. hysterophorus* (33.3%).

Adult longevity was highest when larvae were reared on *T. portulacastrum* (11.2 d) which was at par with those reared on *A. philoxeroides* (11 d). Maximum pre-oviposition period of 5.3 days was observed on adults obtained from larvae reared on *E. hirta* which was statistically at par with pre-oviposition period on *E. crassipes* (4.7 d). Lowest pre-oviposition period of 2.3 days was observed on *T. portulacastrum* reared moths.

The total developmental period from hatching of larvae to emergence of adult was least in *T. portulacastrum* and *R. obtusifolius* (27 d) and highest in *P. hysterophorus* (44.4 d). Total developmental time increased in less preferred hosts. Rani *et al.* (2002) reported prolonged larval period, least survivorship percentage, minimum larval and pupal weight and growth and developmental indices on several less preferred hosts. Sushilkumar and Bhan (1996) reported increase in pre-oviposition time in *Zygogramma bicolorata*, a biocontrol agent of *P. hysterophorus* when reared on the alternative host, *Xanthium strumarium*.

*S. litura* accepted a wide range of host weeds. Mandal and Mandal (2000) also reported feeding preference and life cycle of *S. litura* on different crop and weed plant species. They reported good feeding of *S. litura* on weeds like *Ipomoea aquatica* and *Amaranthus viridis*. Though in our experiment *S. litura* did not feed on *Cynodon dactylon*, Jamjanya and Quinsberry (1988) reported feeding of *S. litura* on some genotypes of *C. dactylon*.

TABLE 1. Development of *S. litura* reared on eight species of common weed hosts

Host weeds	Developmental period (days)			Weight of the immature stages (g)			Per cent survival		Adult longevity (days)	Pre-oviposition period (days)	Total developmental period
	Egg	Larva	Pupa	Larva	Pupa		Larva	Pupa			
<i>A. philoxeroides</i>	3.7 <sup>cd</sup>	26.2 <sup>b</sup>	15.2 <sup>a</sup>	0.1 <sup>c</sup>	0.27 <sup>abcd</sup>		66.7 <sup>b</sup>	46.7	34.6	3.0 <sup>c</sup>	34.6 <sup>c</sup>
<i>E. hirta</i>	5.0 <sup>ab</sup>	26.6 <sup>b</sup>	16.4 <sup>ab</sup>	0.1 <sup>e</sup>	0.25 <sup>bcd</sup>		53.3 <sup>c</sup>	43.3	40.0	5.3 <sup>a</sup>	40.0 <sup>b</sup>
<i>T. portulacastrum</i>	2.3 <sup>e</sup>	16.6 <sup>e</sup>	10.4 <sup>d</sup>	0.9 <sup>b</sup>	0.34 <sup>a</sup>		93.3 <sup>a</sup>	86.7	28.0	2.3 <sup>c</sup>	28.0 <sup>d</sup>
<i>P. hysterophorus</i>	5.3 <sup>a</sup>	32.2 <sup>a</sup>	12.2 <sup>c</sup>	0.1 <sup>e</sup>	0.20 <sup>d</sup>		53.3 <sup>c</sup>	33.3	42.8	3.0 <sup>c</sup>	42.8 <sup>a</sup>
<i>R. obtusifolius</i>	2.7 <sup>de</sup>	17.0 <sup>e</sup>	10.0 <sup>d</sup>	1.1 <sup>a</sup>	0.31 <sup>ab</sup>		86.7 <sup>a</sup>	80.0	29.4	2.7 <sup>c</sup>	29.4 <sup>d</sup>
<i>C. intybus</i>	2.3 <sup>e</sup>	19.2 <sup>d</sup>	12.6 <sup>c</sup>	1.0 <sup>ab</sup>	0.32 <sup>ab</sup>		90.0 <sup>a</sup>	73.3	27.8	2.7 <sup>c</sup>	27.8 <sup>d</sup>
<i>I. fistulosa</i>	4.0 <sup>bc</sup>	23.6 <sup>c</sup>	14.8 <sup>b</sup>	0.4 <sup>cd</sup>	0.28 <sup>abc</sup>		60.0 <sup>c</sup>	36.7	37.8	3.7 <sup>bc</sup>	37.8 <sup>b</sup>
<i>E. crassipes</i>	3.3 <sup>cd</sup>	27.0 <sup>b</sup>	12.2 <sup>c</sup>	0.2 <sup>de</sup>	0.21 <sup>ab</sup>		80.0 <sup>ab</sup>	60	40.0	4.7 <sup>ab</sup>	40.0 <sup>b</sup>
SEm $\pm$	0.4	0.5	0.5	0.1	0.02		4.9	5.0	0.8	0.4	0.8
CD <sub>0.05</sub>	1.2	1.6	1.4	0.2	0.1		14.6	14.9	2.4	1.1	2.4

Mean in the same column followed by the same letter are not significantly different at  $p = 0.05$ .

High preference and short developmental period of *S. litura* on the host weeds showed that though these weeds act as alternative hosts of the insect and negatively affect biocontrol programme the insect plays an important role in checking weed population in field.

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## Studies on the transmission of citrus greening bacterium, *Candidatus Liberobacter asiaticus*, by the vector, citrus psylla, *Diaphorina citri* Kuwayama in Coorg mandarin

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**ABSTRACT:** Citrus greening disease caused by a vectored pathogen, *Candidatus Liberobacter asiaticus*, is known to contribute for the decline of 'coorg mandarin' in most of coffee plantations in Kodagu. To establish the vector role, infective psyllids, *Diaphorina citri* Kuwayama were studied and their salivary glands were subjected to electron microscopic examination. Elongated rod-like structures were found in the salivary gland cells of infective psyllids supporting their vector role. In transmission studies, 18 months after infective feeding, 2 out of 5 test plants developed typical symptoms of greening disease. PCR amplification of DNA with primer pairs F-Las and R-Las revealed presence of citrus greening bacteria in the test plants. © 2007 Association for Advancement of Entomology

**KEYWORDS:** citrus greening, *Diaphorina citri*, salivary glands, transmission

'Coorg mandarin' (*Citrus reticulata* Blanco) is one of the important intercrops in coffee plantations of Kodagu district situated on the eastern slope of the Western Ghats in India. Citrus greening caused by a vectored pathogen, *Candidatus Liberobacter asiaticus*, probably contributed for the decline, apart from Citrus Tristeza Virus and other abiotic factors. Citrus psylla, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae) has been implicated as vector of the greening disease (McClellan and Oberholzer, 1965; Capoor *et al.*, 1974; Catling and Atkinson, 1974; Garnier *et al.*, 1984; Jagoueix *et al.*, 1996). Studies on the population dynamics of citrus psylla in the region revealed very low population throughout the year except during summer

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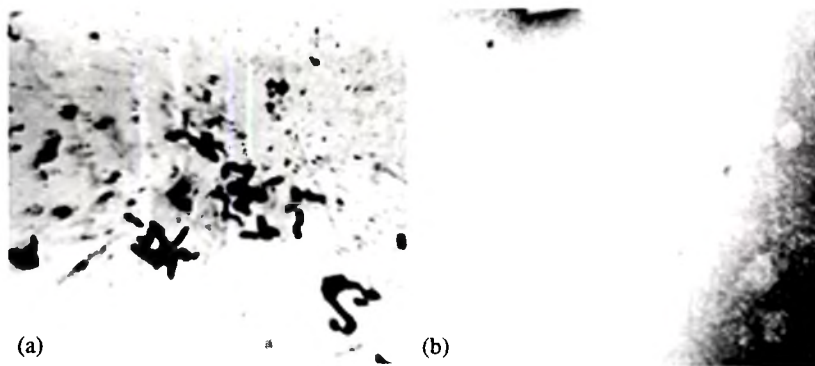


FIGURE 1. Transmission electron micrograph of citrus greening bacteria (a) in salivary gland duct (25000x), (b) in salivary gland cells (76000x)

months. The inconspicuous number and limited flight behaviour of psyllids raised doubts on their decisive role in contributing to mandarin decline in the region. Hence, detection of the bacterium within the vectors by electron microscopic and PCR studies was considered appropriate and sensitive method to confirm their role in transmitting the disease.

Citrus psyllids were reared in the laboratory on the alternative host, curry leaf plant (*Murraya koenigi*), which is not considered as a host for greening pathogen. For acquisition feeding, newly emerged adults were allowed to feed on twigs of Coorg mandarin plants with typical greening symptoms and tested positive in PCR for the pathogen. Acquisition feeding time was recorded once the psyllids were settled on the mid ribs or veins on the leaves. After 48 h acquisition feeding and three days incubation period, infective psyllids were used for electron microscopic, PCR and transmission studies.

**Electron microscopy:** Psyllids after acquisition feeding were dissected out in saline solution, salivary glands were excised, fixed in 3% glutaraldehyde in 0.2M phosphate buffer and post fixed in 1% osmium tetroxide in phosphate buffer. The tissues were dehydrated in ascending series of acetone, infiltrated with acetone and Spurr resin and embedded in pure Spurr resin. Sections (90–120 nm) were cut and stained with 2% uranyl acetate and 2% lead citrate. The sections were observed under JEOL 100 electron microscope.

Elongated rod-like structures were found in the salivary gland ducts and cells of infective psyllids (Fig. 1a). These structures were found in more numbers in the salivary ducts. At higher magnification and at a different plane of section of the salivary gland cell, the bacterium appeared round to elongated shape with multiple layered membranes (Fig. 1b). Morphologically these bodies were similar to the bacterium found in the sieve tubes of infected plants (Garnier and Bove, 1977). The pleomorphic bodies were absent in tissues of non-infective psyllids. Transmission electron micrographs of vector tissues and presence of greening bacterium in them



FIGURE 2. Coorg mandarin seedlings showing greening symptoms, 18 months after infective feeding

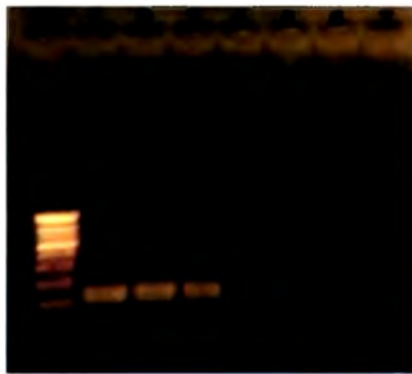


FIGURE 3. Agarose gel electrophoresis of DNA from leaf: Lane M— Marker, Lanes 1, 2— test plants, lane 3— source plant, lanes 4, 5, 6— test plants, lane 7— healthy plant

were in agreement with past observations of Moll and Martin (1973), Chen *et al.* (1973) and Xu *et al.* (1988).

**Transmission to test plants:** Five six-month old ‘Coorg mandarin’ plants, budded on Ragpurlime rootstock, were used as test plants. After acquisition feeding and incubation period, two infective psyllids each were transferred to the test plants. Infection feeding of at least 12 h was ascertained as was done for acquisition feeding. After the infection feeding the psyllids were recovered for PCR studies. Healthy psyllids reared on curry leaf plants without acquisition feeding released on to Coorg mandarin plants of the same age served as control. The plants were grown under insect proof cages with recommended doses of fertilizers and regular irrigation. Observations were recorded on symptom expression at regular intervals. Eighteen months after feeding by infective psyllids, two out of five seedlings developed typical symptoms of greening as observed in the field, viz. sparse yellow foliage with

irregular patches between the main veins, mottling resembling zinc deficiency and stunted growth (Fig. 2). The control plants were devoid of any such symptoms. These results confirmed that a pair of psyllids with 48 h acquisition feeding with three days incubation period and 12 h of infection feeding could efficiently transmit the pathogen to the test plants.

**PCR studies:** Leaf midribs of test plants, source plants on which the psyllids were exposed for acquisition feeding and healthy plants were homogenized separately for DNA extraction. The extracted DNA was amplified as per the protocol specific for Asian strain of greening by Hung *et al.* (2004). The resultant amplified DNA samples were electrophoresed along with suitable marker in 1% agarose gel containing ethidium bromide using TBE buffer. The primer F-Las and R-Las amplified 226bp fragment specific for *L. asiaticus* from the samples of test plants (Fig. 3). Similar results were given by DNA of the samples of diseased plants on which the psyllids were exposed for acquisition feeding. Similar results on the presence of the pathogen in psyllids were reported from Central and North-Eastern India (Das, 2004 Das *et al.* 2007). No amplification was obtained from DNA samples extracted from the healthy plant.

The present study confirmed the vector role of psyllids in transmission of citrus greening pathogen and reinforces the necessity of vector management practices for minimizing the subsequent spread of the disease in the region.

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## Male contribution of lipids to the female silkmoth *Bombyx mori* L., during mating

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**ABSTRACT:** The lipid content in reproductive tissues of virgin and mated male and female moths of *Bombyx mori* L. was determined. It was also determined after egg laying in both virgin and mated female moths. Lipid content decreased in the reproductive tissues of male moth after mating. In female, contrary to male, a significant rise was observed in all the tissues. Following oviposition the lipid content decreased in all the tissues of both virgin and mated females; however, the loss was more in mated moths. It is concluded that the male moth contributes lipids to the female moth at the time of copulation. © 2007 Association for Advancement of Entomology

**KEYWORDS:** *Bombyx mori*, mating, oviposition, lipid

Studies on various insects have shown that males not only contribute sperm to the female during mating but also some substances secreted by different tissues of reproductive system (Leopold, 1976; Friedel and Gillott, 1977) and nutrients via spermatophore and seminal fluid (Thornhill, 1976; Boggs and Gilbert, 1979). This is of great importance to the reproductive success, since seminal fluid components perform diverse functions like induction of oogenesis, enhancement of fertility and fecundity, suppression of remating, stimulation and acceleration of oviposition, etc. in the female and benefits both the male and its mate (Pickford *et al.*, 1969; Gillott, 1988; Wolfner, 1997). In many insects, females make use of the seminal fluid as a source of nutrients for somatic maintenance or egg production or egg deposition (Boggs and Watt, 1981; Marshall, 1985). Among nutrients, lipids are of vital importance as metabolic reserves especially for those insects which exhibit prolonged periods of metabolic activity like embryogenesis (Gilbert, 1967) and mating (Ranganathan and Padmanabhan, 1994). There exists a lacuna in understanding the nutrient utilization, especially the lipids and their source, at the time of mating and egg laying in silkmoth *Bombyx mori* L. and hence this work.

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TABLE 1. Lipid content in tissues of male silkmoths before and after mating

Reproductive tissues	Lipid content (mean $\pm$ SE) mg/g	
	Virgin moths	6 h mated moths
Testes	78.85 $\pm$ 1.04	33.55 $\pm$ 1.12*
Vas deferens	64.57 $\pm$ 0.37	40.20 $\pm$ 0.33*
Seminal vesicle	69.19 $\pm$ 1.09	52.80 $\pm$ 1.06*
Ejaculatory duct	32.41 $\pm$ 0.67	24.35 $\pm$ 0.66**
Accessory gland	48.59 $\pm$ 0.37	28.50 $\pm$ 0.31*

Significant at the level of \* 0.001, \*\* 0.005

TABLE 2. Lipid content in tissues of female silkmoths before and after mating

Reproductive tissues	Lipid content (mean $\pm$ SE) mg/g	
	Virgin moths	6 h mated moths
Ovary	43.60 $\pm$ 0.69	65.90 $\pm$ 1.04*
Oviduct	14.79 $\pm$ 0.75	25.22 $\pm$ 0.88*
Spermatheca	3.99 $\pm$ 0.01	6.01 $\pm$ 0.11*
Bursa copulatrix	7.25 $\pm$ 0.02	15.90 $\pm$ 0.23*
Accessory gland	11.31 $\pm$ 0.51	22.39 $\pm$ 1.01*

Significant at the level of \* 0.001

Silkworm seed cocoons (NB<sub>4</sub>D<sub>2</sub>) were sexed and maintained in separate cages to avoid copulation immediately after emergence. The female moths were divided into two sets. The first set was allowed to lay eggs as virgins. The second set females were allowed to mate with male moths for six hours. Mated females were divided into two groups. The first group females and mated male moths of second set were used for the assay immediately. The second group females were allowed to lay eggs. After egg laying the virgins of the first set and mated females of second group were used for the assay. The virgin male before mating and virgin female before egg laying were also used for lipid assay.

The moths were dissected in silkmoth saline (Yamaoka, 1977) and tissues of reproductive system were pooled from ten moths. The lipid assay was done following the method of Handel (1985). The data generated by repeating the experiment thrice were analyzed by students 't' test (Snedecor and Cochran, 1994).

In virgin male moths the lipid content was more in testes followed by seminal vesicle, vas deferens, accessory glands and ejaculatory duct (Table 1).

After mating it decreased in all these tissues. The maximum loss was in testes (57%), followed by accessory reproductive glands (42%) and the least was in seminal vesicle (24%).

TABLE 3. Lipid content in tissues of virgin and mated female silkmoths after egg laying

Reproductive tissues	Lipid content (mean $\pm$ SE) mg/g	
	Virgin female	Mated female
Ovary	17.87 $\pm$ 0.28	23.31 $\pm$ 0.36*
Oviduct	11.09 $\pm$ 0.56	10.10 $\pm$ 0.51 NS
Spermatheca	3.78 $\pm$ 0.03	2.09 $\pm$ 0.06*
Bursa copulatrix	6.86 $\pm$ 0.02	5.53 $\pm$ 0.08*
Accessory gland	8.57 $\pm$ 0.38	8.39 $\pm$ 0.38 NS

Significant at the level of \*0.001; NS, Not significant.

In the reproductive tissues of the female moths the lipid content increased following mating (Table 2). The gain was relatively more in bursa copulatrix (119%) followed by accessory reproductive gland (98%), ovary (51%), and spermatheca (51%).

Following oviposition the lipid content decreased in all the tissues of both virgin and mated moths (Tables 2 and 3). The loss was comparatively more in mated moths.

In male moth the loss of lipids can be attributed to the seminal flow into female during copulation. Testis being the major loser, the loss may be due to the outflow of sperms from the testis. Vas deferens and ejaculatory duct transport the sperms from testis to seminal vesicle and from there to bursa copulatrix of female respectively and exhibit peristaltic contractions to propel the sperms down (Romoser, 1973). Ejaculatory duct also secretes some of the substances which are introduced into the female (Gillott and Friedel, 1977). In these tissues the loss may be due to lipolysis to provide energy for muscle contraction and may also aid in secretion. The seminal vesicle stores the sperms until they are delivered to female and the nourishment of sperms in seminal vesicle may be supported by the lipids. The accessory gland is a site of many functions like activation of sperms, production of spermatophores, nourishment of sperms, etc. which may utilize the lipids for these functions. Accessory glands are also known to secrete some of the substances which are transferred to female where they perform several functions (Rockstein, 1964; Thornhill, 1976; Wolfner, 1997). In *Odontopus varicornis* tri-acyl glycerol, a lipid from the accessory gland contributes to the energy needs for mating especially in prolonged mating (Ranganathan and Padmanabhan, 1994). This may be true in *Bombyx mori* as well because the lipid depletion was more in the accessory glands. The depletion of lipids in some of the reproductive tissues like oviduct, bursa copulatrix and accessory glands of female may likewise be due to its utilization for energy requirements.

As the lipid level increases in females and decreases in male following mating it may be assumed that the male supplies lipid to the female during mating. This male contribution may be either direct or indirect by mobilization of nutrients from reproductive tissues as well as non reproductive tissues (Boggs and Gilbert, 1979; Marshall, 1985).

It is concluded that in *B. mori* the male moth contributes nutrients for the post mating activities of the non feeding female moth such as oviposition. In providing nutrients, it appears that the male makes an investment on female to ensure viable progeny of its own.

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Nair M. R. G. K. and Mohandas N. (1962) On the biology and control of *Carvalhoeia arecae*, a pest of areca palms in Kerala. *Indian Journal of Entomology* 24: (1) 86–93.

Jalaja M. Muraleedharan D. and Prabhu V. K. K. (1973) Effect of extirpation of median neurosecretory cells on reproduction in the female red cotton bug, *Dysdercus cingulatus*. *Journal of Insect Physiology* 19(1): 29–36

Books and Articles in Books:

Novak V. J. A. (1966) *Insect Hormones*. Methuen and Co., 478 pp.

Wigglesworth V. B. (1964) The hormonal regulation of growth and reproduction in insects. In: *Advances in Insect Physiology* Vol. 2 (Eds. Beament J. W. L., Treherne J. E. and Wigglesworth V. B), Academic Press, London, pp 247–335.

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